State of California

Memorandum

To: Richard Ayalya, Registration Specialist
Pesticide Registration Branch

Date: July 27, 1995

Place: Sacramento

445-4262

From: **Department of Pesticide Regulation** James R. Sanborn, Staff Toxicologist
Worker Health and Safety Branch

HSM-95004
(No. assigned after issuance of memo)

Subject:

PRODUCT NAME: Bravo®
ACTIVE INGREDIENT: Chlorothalonil
COMPANY NAME: ISK Biosciences Corporation
I.D. NUMBER: RA-126116-E
DOCUMENT NUMBER: 275-170
EPA REGISTRATION NUMBER: 50534-0-

1. Study to evaluate the urinary metabolites of chlorothalonil following dermal application to male rhesus monkeys.
2. Development of method for monitoring exposure of workers to ASC 2787 (chlorothalonil): Extraction of 5-chloro-2,4,6-(trismethylthio) isophthalonitrile from human urine (on p. 3)

Four male rhesus monkeys (weight range 3.5-5.0 kg) were treated with mixtures of Bravo® 720 and ^{14}C-chlorothalonil (radiochemical purity 96.3%; specific activity 124.5 mCi/mmole) over a surface area of 180 cm^2 at a dose rate of 4.79 ± 0.05 mg/kg (128.1 ± 11.2 ug/cm^2, 0.5 ml/kg of Bravo® formulation) and an average radioactive dose of 4.5 X 10^{-8} dpm per animal. An additional animal was treated only with vehicle control and sacrificed at the end of 48 hrs. The treated area was covered with a non-occlusive patch. Urine and feces were collected for the analysis of radioactivity. After exposure of the animals for 48 hours, the treated area was washed and two of the test animals were sacrificed. The two remaining animals were held for a total of 120 hrs for the purposes of collection of additional urine and fecal samples.

From the sacrificed animals, in addition to the urine and feces, blood, liver, kidney, intestine, non occlusive patch, skin washings and the treated area were analyzed for radioactivity. The "skin washing" was accomplished by rinsing the treated area twice with approximately 100 ml acetone. This is **not** the conventional method for skin washing. The generally accepted procedure involves washing the treated skin area with a surfactant/water mixture that mimics the type of soap/water solution that a worker might use during bathing. Following this acetone washing, the excised skin was blended in methanol twice and then a final blending of the skin in acetone was carried out to ensure that all solvent extractable radioactivity was removed from the skin. Following this exhaustive solvent washing/extraction, the skin was oxidized and counted scintillation to estimate bound ^{14}C-residues.

After acidification with HCl to pH 2, the urine was extracted with ethyl acetate and then the extract was treated with diazomethane to methylate polar species for subsequent gc/ms analysis.

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Recovery of Radioactivity

The data discussed below are expressed in means for both sacrifice groups (48 and 120 hrs) as there was not any time dependence on the recovery of radioactivity from any of the matrices.

Skin and Patch

The total amount of recovered radioactivity averaged 94.8 ± 4.3% with most of the recovered radioactivity observed in the non-occlusive patch (81. 0 ± 4. 0% of administered dose) which covered the treated area for the first 48 hrs. Another 8.4 ± 1.5% of the administered radioactivity was found in the acetone skin washings.

Urine/Feces

The urinary and fecal elimination as a percent of applied dose were 1.0 ± 0.3 and 1.0 ± 0.3, respectively. Analysis of the urine of the extracts with diazomethane did not provide levels of methylated mono-, di- or trithiols that were above the limit of detection.

Percent Absorbed

This document suggests that the percent dermally absorbed dose should be determined from the combined sum of the eliminated dose (urine+ feces), skin (solvent extractable + bound radioactivity) and tissue residues. The value that was obtained for the sum of these matrices was 5.4%. The table below itemizes the location of the radioactivity that was utilized to calculate the absorbed dose.

Radioactivity in various matrices used to calculate dermal absorption in rhesus monkeys

Matrix	Percent (SD)[a/]
Urine	0.97 (0.25)
Feces	1.04 (0.34)
Tissues	0.26 (0.26)
Skin	3.08 (0.87)
Total	**5.35**

a/ Table 2, p.40, DPR Reg. Doc. No. 275-170

Discussion of Monkey Study

Since a large portion (76-85%) of the applied dose was recovered in the non occlusive patch that covered the treated area, the-value of 5.4% for a dermal penetration study in this primate is probably not representative of the dermal penetration value for chlorothalonil. Since the vapor pressure of this fungicide is less than 10^{-6} mm Hg, it is not obvious how the radiolabeled fungicide moved from the treatment area to the occlusive patch. From the standpoint of worker exposure to this fungicide, similar transference to clothing as occurred to the occlusive patch during this monkey study would not be expected to occur in the field.

Conclusion

From the above discussion, it is clear this study was not well executed. Therefore, the percent dermal absorption, even though it is from a primate, will not be used in worker exposure estimates.

2. Development of method for monitoring exposure of workers to ASC 2787 (chlorothalonil): Extraction of 5-chloro-2,4,6-(trismethylthio) isophthalonitrile from human urine.

The purpose of this study was to examine the extraction efficiency from urine of the chlorothalonil metabolite, 5-chloro-2,4,6-(trismethylthio) isophthalonitrile. Human urine was first fortified with this metabolite at 10 ug/liter and then the samples were extracted with either ethyl acetate or dichloromethane. There was no pH effect on the extraction efficiency. These extracts were loaded on a C-18 Sep-Pak® and then methanol was used to elute the metabolite from the column. Since the range of recoveries for this procedure was 25-30%, it cannot be considered an acceptable method for extracting chlorothalonil metabolites from urine.

Discussion of Monitoring Method

This very preliminary method for estimation of urinary metabolites of chlorothalonil must be placed in the context of the preceding discussion of the dermal penetration of this fungicide in rhesus monkeys where no metabolites containing one, two or three mercaptans were found at a dose of 4.8 mg/kg. Perhaps, because these metabolites constitute a very low percentage of the radioactivity in urine and urinary excretion is slightly less than 1% of the applied dose, it is not unexpected that they were not found in the preceding study with the monkeys.

cc:
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